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Biocatalysis combined with physical technologies for development of a green biodiesel process

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ABSTRACT

Biodiesel derived from the transesterification of vegetables oils or animal fats with alcohol is composed of saturated and unsaturated long chain alkylesters. The process has some technical problems that must be resolved to reduce the high cost of operation. Limitation of mass and heat transfers, reaction equilibrium, batch mode operation and product purification affects conversion yield, time of reaction, productivity and energy cost. This paper highlights some recent advances in process innovation for the biodiesel industry to develop a sustainable continuous process, environmentally benign and cost effective. Eco-friendly physical technologies as microwave, ultrasound and membrane reactors and their possible combination have successfully improved the enzymatic transesterification for biodiesel production.

Contents

1. Introduction

The world trend of sustainable technologies development has contributed to green chemistry emergence, which directed the responsibility of the scientific community toward developing new, improved and sustainable industrial processes. For such a trend, biofuels are considered mainstream due to energy importance, environmental pollution and necessity of fossil fuel substitution. Biodiesel, or fatty acid alkyl esters, has been considered an environmentally friendly alternative fuel for diesel engines [1]. It has some advantages like greenhouse gas emissions reduction, use of renewable sources and applicability in the existing transport sector [2].

Commercial biodiesel is produced by transesterification of vegetable oils and animal fats with methanol or ethanol on stirred tank reactors in the presence of base or acid catalysts. This process has some operating problems which include time of reaction, energy consumption and low productivity that contributes for increasing the production cost. For solving these issues, the biodiesel researches have focused on developing process intensification technologies [3]. These studies propose some eco-friendly technologies for reducing operational costs and simplifying the continuous process of the biodiesel production in large scale. Technologies such as microwaves, ultrasound, enzymatic catalysis and membrane reactors have been studied recently (Fig. 1) and used in combined mode with promising results.

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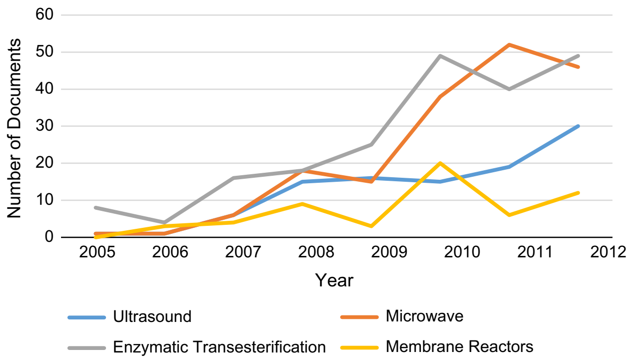


Fig. 1. Number of documents published for biodiesel production [115].

Microwave radiation has been used as an alternative heating method which showed more efficiency in all catalytic models, generating a faster reaction activation energy compared to conventional heating. As a consequence, short reaction times with a reduced energy consumption are obtained [4]. Besides it, microwave reactors have been developed for continuous mode operation.

Considering the immiscible problem between alcohol and oil, ultrasonic irradiation could be an alternative tool for increasing the mass transfer of liquid–liquid heterogeneous systems [5]. With increased liquid–liquid mass transfer, oils and methanol are easily emulsified, and cavitation contributes to the catalytic reaction. Under ultrasonic irradiation, the transesterification can be carried out at a low temperature, and smaller amounts of catalyst and alcohol are needed. Ultrasound, as well as microwaves, showed good performance with heterogeneous, homogeneous and enzymatic catalysis.

In terms of catalysis, the enzymatic transesterification, considered as a green process due to biochemical characteristics of the biocatalyst, presents some advantages like compatibility with variations in the feedstock quality, fewer process stages (heterogeneous catalysis), higher quality glycerol, easy phase separation (no emulsification from soaps), reduced energy cost and wastewater volumes [2]. Nevertheless, the reaction time of enzymatic transesterification is still much longer than homogeneous catalysis and for that reason it is considered an important research target in biodiesel process development.

Although the major biodiesel production cost is due to feedstock, the downstream process for biodiesel purification is considered expensive. The washing step to remove free glycerol, soap, alcohol excess and residual catalyst uses a large volume of water, generating a wastewater stream that must be treated [6–8]. The multiple downstream processes expends time and require additional cost [9]. The intensification technologies comprise the application of novel reactors or coupled reaction–separation processes combined to optimize the reaction rate, reduce the residence time and the number of operation units in whole process [3,9]. In membrane reactors, the simultaneous reaction and separation step not only reduces the unit operations but also avoids the reaction equilibrium limitation, besides the possibility of application in continuous mode.

This work presents some advantages and recent advances on the application of microwave, ultrasound, enzymatic catalysis and membrane reactors and its combination for overcoming the technical limitations in conventional biodiesel processing like mass and heat transfer; reaction equilibrium and purification. This process integration are useful for the implantation of a continuous mode operation as a sustainable process, due to its simultaneous environmental safety and operational cost reduction.

2. Microwave-assisted process

It has been reported in biodiesel transesterification that yield and reaction time vary greatly when heating by microwave irradiation instead of using conventional heating methods [10–14]. The selective heating of microwave irradiation requires less energy compared to conventional heating methods. It is possible to perform reactions faster, efficiently and safely using a microwave apparatus [15,16]. Besides, it is considered to be a sustainable technology due to its environment-friendly characteristic and its reduced energy consumption.

Microwaves are non-ionizing radiations *i.e.*, electromagnetic waves of low energy that which cannot ionize the atoms crossed. In spite of its induction molecular agitation capacity, such as ion migration or dipole rotation; microwaves cannot change the molecular structure [17]. This results in molecular attrition and collisions, which generates localized heating and thereby accelerating the chemical reaction, giving high conversion in a short time [14]. Compared with conventional heating, microwave irradiation requires less energy when solvents with higher dielectric loss factors are used [15,18]. According to this principle, the microwave heating has been studied for substitution of conventional heating in biodiesel production.

Microwave irradiation can be applied with different kinds of catalysts and feedstock, generating a satisfactory yield conversion and reaction time. Besides the homogeneous catalysts have the best conversion results, heterogeneous catalysis has showed same performance with microwave heating. It has the advantage of an easy catalyst separation step, which could reduce the operation cost and the number of unit operations in the whole process. Zhang et al. [19] developed an efficient microwave-assisted transesterification approach to produce biodiesel from yellow horn (*Xanthoceras sorbifolia* Bunge.) oil with a heteropolyacid (HPA) catalyst, namely, $Cs_{2.5}H_{0.5}PW_{12}O_{40}$. The optimization study obtained the maximum yield of fatty acid methyl esters (FAME) of 96.22% in 10 min using temperature of 60 °C, molar ratio of methanol:oil; 12:1, catalyst concentration of 1% (w/w of oil) and minimum recycle number of nine times. These results showed that the microwave method was more efficient compared to conventional heating.

Hsiao et al. [12] evaluated another heterogeneous base catalyst on the microwave-assisted transesterification of soybean oil. The results showed that nanopowder calcium oxide (nano CaO) combined with microwaves were greater than conventional heating in soybean oil transesterification. The results achieved 96.6% of conversion in 60 min using a methanol/oil molar ratio of 7:1, 3.0 wt% catalyst, and a reaction temperature of 60 °C.

Chen et al. [20] described the influence of microwave power on transesterification of waste-cooking-oil biodiesel. They discovered that conversion yields raises with reaction power. However, the high power microwave could damage organic molecules. The optimal reaction conditions were 0.75 wt% CH_3ONa catalyst, a methanol-to-oil molar ratio of 6, a reaction time of 3 min, and energy power of 750 W.

Microwave irradiation also has been applied in simultaneous extraction and reaction like the transesterification of algae oil, which has intracellular oil accumulation. Patil et al. [21] studied the application of microwave irradiation on the simultaneous extraction and transesterification (*in situ*) of dry algal biomass. The results achieved a high oil/lipid extraction yield from dry algal biomass and an efficient conversion to biodiesel simultaneously in optimal conditions: dry algae to methanol (wt/vol) ratio of 1:12, catalyst concentration about 2 wt%, and reaction time of 4 min.

Although high performance reported, microwaves need to be applied in continuous mode for its industrial approach. The continuous-flow process of biodiesel using commercially-available

Table 1
Resume of recent studies of microwave-assisted transesterification process for biodiesel production.

Reference	Catalyst/oil	Temperature (°C)	Yield (%)	Reaction time (min)
[11]	Alkali/rapeseed oil	40–50	88.3–93.7	3–5
[110]	KOH/dry algal	190	26.3	10
[111]	Sodium potassium tartrate doped zirconia/soybean oil	65	97.29	30
[112]	Acid and alkali/*Jatropha* seed	–	97.29	48
[19]	Heteropolyacid/yellow horn oil	60	96.22	10
[12]	nanopowder calcium oxide/soybean oil	65	96.6	60
[113]	NaOH/macroalgae oil	–	–	3
[13]	NaOH/waste palm oil	–	97%	0.5

scientific microwave equipment offers a fast, easy route to study the capacity and reaction performance. The operation permits the system reaction under atmospheric conditions and use of new or used vegetable oil. Energy consumption calculation suggests that the continuous-flow microwave process for the transesterification reaction is more energy-efficient than using conventional heating equipment [22].

The recent research on microwave-assisted transesterification shows its efficient application on continuous processes with minor energy consumption and faster reaction than conventional heating. It seems to be a robust technology due to the possibility of application with heterogeneous catalysis and with any raw material. Moreover, microwave heating is considered an environmentally friendly process. The summary of results from recent studies using microwave-assisted transesterification process for biodiesel production is presented in Table 1.

3. Ultrasonic-assisted process

Ultrasonic irradiation can induce an effective emulsification with improved mass transfer in biodiesel transesterification so that the rate of ester formation under ultrasonic mixing conditions is higher than traditional stirring conditions [23–26]. Research indicated that ultrasonic mixing is efficient, faster and economically functional with many advantages compared to the conventional stirring [5,26,27]. It is considered a green process since it is an eco-friendly technology with environmentally harmless.

The ultrasonic technique has been used for the transesterification reaction in biodiesel production aiming on improvement of yield conversion, reaction time and energy consumption reduction. Recent studies have shown that ultrasound could substitute the conventional stirring method and be combined with homogeneous or heterogeneous catalysis with its application on continuous mode operation.

Ultrasound has initially been applied in homogeneous catalysis with successful results by Thanh et al. [28] that produced biodiesel from canola oil with methanol in basic catalysis at room temperature, using a low frequency ultrasound (20 kHz) obtaining a conversion of 99% in 50 min in optimal conditions and Santos et al. [29], who studied the optimization of soybean oil methanolysis under ultrasound irradiation in batch mode and observed that the methanol to oil ratio showed major positive effect in the reaction than the catalyst concentration, which could be related to reaction equilibrium deviation and mass transfer limitation.

Despite high conversion yield, the homogeneous system needs a catalyst separation step, which could be simplified in heterogeneous catalysis. Kumar et al. [30] performed a transesterification assisted by ultrasonication under atmospheric conditions. The solid catalyst (Na/SiO_2) and ultrasound reduced the reaction time compared to the conventional batch process, resulting in 98.53% biodiesel conversion in 15 min, achieved in optimal conditions of molar ratio oil to methanol 1:9, catalyst concentration of 3 wt% of oil.

Industrial design have developed and converted batch for continuous process due to some benefits of productivity. Batch mode operation presents some limitations generated by reaction equilibrium and mass transfer resistance. Thanh et al. [31] performed an alkaline alcoholysis of waste cooking oils (WCO) with methanol in a continuous ultrasonic reactor of low-frequency; 20 kHz, in a two-step solvent addition. The FAME yield was extremely high even at the short residence time in the ultrasonic reactor (less than 1 min for the two steps) at room temperature. Using the approach of two-step solvent addition, the reaction rate was faster with small alcohol concentration. The authors considered that conversion rates are faster with small reagent concentration, generating a better performance of catalyst in a continuous process.

Although ultrasound utilization is related to mass transfer improvement, another recent application has presented the ultrasound usability in process control, with the same principle of sonar. For the biodiesel process, during the alcoholysis reaction, the separated glycerin sediments at the bottom and forms a heterogeneous phase. Low-intensity ultrasound pulse echoes use the density of glycerin and methyl ester difference to measure the time of flight (TOF) of a sound wave, which gives information on the glycerin separation situation during the reaction. Monitoring the variation of glycerin over time can be used to determine the glycerin separation start time, glycerin separation rate or separation end time. It can be used for establishing the reaction end or analytical determination of various parameters on transesterification reaction [32,33].

The effects of high-intensity ultrasound irradiation and temperature on glycerin separation start time and separation rate during the soybean oil transesterification were studied by Koc and McKenzie [33]. They evaluated the ultrasonication time and temperature on glycerin separation start time and separation rate (responses measured by ultrasound monitoring). The conditions that obtained the lowest starting times for glycerin separation were reaction temperature of 50 °C, ultrasonication of 5 min and ultrasonication rate of 90%.

The most recent works published for application of ultrasound in transesterification to biodiesel has focused on utilization of new heterogeneous catalysts and new sources of non-edible oils. Choedkiatsakul et al. [34] investigated the application of calcium oxide (CaO) and potassium phosphate (K_3PO_4) and Badday et al. [35] applied a gamma alumina supported tungstophosphoric as heterogeneous catalysts. Guo et al. [36] performed the alcoholysis in the presence of a Brønsted acidic ionic liquid-based catalyst, demonstrating the versatility of ultrasound technique combined with various catalysts. The same characteristic is observed for the types of raw materials used: Manh et al. [37] used different blends of tung, palm and canola oils; Encinar et al. [38] used castor oil and Paiva et al. [39] used babassu oil.

The ultrasound technique showed its adequation for biodiesel process presenting vantages compared to conventional stirring as efficiency of emulsification and energy input reduction; besides its

Table 2
Resume of recent studies of ultrasound-assisted transesterification process for biodiesel production.

Reference	Catalyst/oil	Frequency (kHz)	Yield (%)	Time reaction (min)
[114]	KOH/soybean	611	90	30
[39]	Alkali/babassu palm		97	10
[15]	Alkali/*Pongamia pinnata*	60	96–97	5
[31]	KOH/Canola	20	99	50
[28]	KOH/waste cooking	20	93.8	56
[35]	Tungstophosphoric acid/ *Jatropha* seed		84	50
[30]	Na–SiO_2/*Jatropha curcus*	24	98.53	15

fitness with all kinds of feedstock, catalyst and operation modes. Ultrasound also is considered as a green process due to its harmless behavior for environment.

The summary of recent studies using an ultrasound-assisted transesterification process for biodiesel production can be observed in Table 2.

4. Enzymatic catalysis

Biocatalysis has been considered a trend for sustainable synthesis technology due to biologic origin of the catalyst, selectivity and the possibility of reusing agro-industrial residues for biocatalyst production, which classifies the method as a green process. Enzymatic catalysis has been applied for biodiesel which starts its industrial scale operation in China. This is the first industrial scale with lipase (EC 3.1.1.3) as the catalyst [40]. These enzymes are obtained from most fungi and bacteria fermentation such as *Rhizomucor miehei*, *Rhizopus oryzae*, *Candida antarctica*, *Candida rugosa*, *Pseudomonas cepacia* and *Thermomyces lanuginosa*, but the commercial immobilized lipase B from *C. antarctica* (Novozym 435) is the most commonly used enzyme and the most expensive catalyst available on the market [41]. Lipase could be immobilized on solid supports both extra- or intra-cellular forms [42]. For that reason, it is considered a heterogeneous catalyst and could simplify the catalyst separation step, beyond the possibility of lipase recycling and application in continuous mode operation. It has been applied for biodiesel synthesis in free- and solvent systems as ionic liquids [43], organic co-solvents [44], supercritical fluids [45] and most recently with glymes [46].

Enzymatic transesterification eliminates the disadvantages of the alkali process by generating the product with high purity, with few downstream operations [47]. It has been reported in the literature that enzymatic transesterification of lipids for biodiesel production is considered technically feasible but the high cost of lipases is the main limitation for a commercially feasible enzymatic production of biodiesel. The technology to re-use enzymes is not enough to be competitive. However, literature data documenting the enzymatic biodiesel productivity together with the development of new immobilization technologies indicates that enzyme catalysis can become cost effective compared to chemical processing [2].

Recent studies have been focusing on improving catalysis performance and stability of the enzyme with aiming on reducing the lipase cost in the biodiesel conversion process. Some different approaches have been developed for application mode of lipases. Solid fermented, whole-cell biocatalyst and immobilized lipase in different supports are the mainly studied modes.

The application of fermented solid was created for reduce cost in lipase production and could be used as a catalyst in batch and continuous operation [48,49]. The solid fermentation of agricultural residues permits that enzyme have a low price compared to commercial enzymes due to its application as fermented solid avoids the extraction, purification and immobilization steps in enzyme production with satisfactory catalytic results in transesterification reaction [49]. Moreover, this approach has potential as a sustainable solution due to utilization of residues from the feedstock for catalyzing the biodiesel synthesis.

Salum et al. [49] produced a solid fermented lipase from *Burkholderia cepacia* LTEB11 in solid fermentation of sugarcane bagasse and sunflower seed meal that was used to catalyze the biodiesel synthesis in a fixed-bed reactor. The results showed a high conversion of 95% after 46 h, obtained at 50 °C, with an alcohol:oil molar ratio of 3:1, in two steps alcohol addition.

Using the same approach, Liu et al. [50] produced a *B. cenocepacia* solid fermented lipase and used it in soybean oil ethanolysis with *tert*-butanol as co-solvent. It was obtained in optimum conditions a highest biodiesel conversion of 86% in 96 h, 45 °C reaction temperature, 200 rpm speed rate, 4:1 alcohol/oil molar ratio, 1.5 wt% fermented solid concentration (based on oil weight, g), 40% *tert*-butanol concentration (based on oil volume, v/v) and 5 wt% moisture content (based on oil weight, g).

The whole-cell biocatalyst is an another approach studied. It is produced by expression of the enzyme on the microbial cells surfaces; intracellular lipase bacteria or fungi and/or different immobilization techniques of fungal mycelium in different supports for continuous mode operation. This approach permits the application of intracellularly-accumulated lipases as whole-cell biocatalysts which avoids the complex procedures of extraction, purification, and immobilization in lipase production process [51]. There are several recent works reporting the utilization of bacteria, yeast and fungi as whole-cell biocatalysts in biodiesel process [52–56].

Genetic engineering has been applied for modeling and has developed new catalysts by cloning and expressing lipase gene in different fungi and bacteria with aim to improve the catalytic activities and stability. The recombinants microbial are used as whole-cell biocatalyst in transesterification reaction immobilized in different supports in continuous reactors.

Gao et al. [54] developed a recombinant *E. coli* expressing novel alkaline lipase-coding gene from *Proteus* sp. for olive oil methanolisys which reached a conversion yield of 100% in 12 h reaction, temperature of 15 °C, which was the lowest temperature catalysis in biodiesel transesterification.

Adachi et al. [57] developed an *Aspergillus oryzae* whole-cell biocatalyst by expressing the lipase gene of *C. antarctica* lipase B (r-CALB) with high esterification activity. The two step reaction consisted first in a hydrolysis of soybean and palm oils using a *C. rugosa* lipase and then submitted to esterification with immobilized r-CALB catalysis which achieved a methyl ester content of more than 90% after 6 h with the addition of 1.5 M methanol. The r-CALB maintained a 90% methyl ester content even after the 20th batch.

Another *A. oryzae* whole-cell biocatalyst developed by Adachi et al. [58] co-expresses two lipase genes in the same cell to improve biodiesel transesterification: a *Fusarium heterosporum* lipase (*FHL*) and a mono- and di-acylglycerol lipase B (*mdlB*). The results obtained showed the best performance of reaction using the lipase-coexpressing whole-cell compared to lipase-mixing method and two step reactions, reaching the best conversion rate and the best ester concentration (98%).

Yan et al. [59] also developed a biocatalyst by expression of two synergistic lipases, *C. antarctica* lipase B and *Thermomyces lanuginosus* lipase on the *Phichia pastoris* cell surface. Results showed a high conversion rate (95.4%) and good operational stability.

Among several modes of application, the immobilization remains the most studied one for enzymatic catalysis in biodiesel process (Fig. 2). Several solid materials, such as ceramics, kaolinites, silica, cellulose, polymers, zeolites and mesoporous matrixes

have been used as support for enzymes immobilization [60–63]. Moreover, several methods have been studied for enzyme immobilization: adsorption; covalent binding; cross-linking and containment behind a barrier (micro-encapsulation, entrapment and confinement). The immobilization merit relies to the activity and the stability preservation of lipase [64].

The packed-bed reactor (PBR) generally leads to higher productivity than a continuous stirred-tank reactor. Moreover, the volumes are reduced, the technology is less expensive (no mobile parts) and enzyme support attrition is avoided [65–67].

Hama et al. [68] created a packed-bed reactor (PBR) system with a fungus whole-cell biocatalyst. Immobilized in polyurethane foam biomass support particles (BSPs), lipase-producing *R. oryzae* cells were cultivated in an airlift bioreactor. The soybean oil methanolysis reached the highest ester content of 90% maintaining around 80% after the 10th cycle.

Wang et al. [69] developed a biodiesel process based on soybean oil methanolysis in a packed bed reactor system using a lipase–Fe_3O_4 nanoparticle biocomposite catalyst obtaining a 88% conversion rate for 192 h, decreasing to approximately 75% after 240 h of reaction.

The application of lipases immobilized in PBR could be linked in continuous downstream separation. Hama et al. [70] created a packed-bed reactor on a bench scale which allows the separation of glycerol byproduct in continuous process. To separate soluble glycerol present in the biodiesel, adsorptive purification using ion-exchange resin was applied to the PBR system. The optimization discovered that the PBR could operate for a long time generating high methyl ester content and an efficient glycerol removal. Hence, this developed model incorporating the enzymatic PBR and glycerol separating system is promising for practical biodiesel production.

The glycerol separation is considered in some new approaches as Xu et al. [71] which developed a two-stage enzymatic ethanolysis in a packed bed reactor using an experimental immobilized lipase (NS 88001) and Novozym 435 to perform reaction of transesterification (first step) and esterification (second one) respectively. The reactions were carried out in a simulated series of reactors considering the separation of the glycerol and water between passes in the first and second stages. Recent advances in enzyme catalysis for biodiesel indicate that some approaches have been tested for application processes in continuous operation. However, some authors considered that the use of the enzyme in the soluble form has lower cost since immobilization process is more expensive [72]. In order to reuse for several cycles, increasing the profitability of the biocatalyst; recent studies are focusing on new materials and new methods that are able to decrease the cost and increase the efficiency and capacity of lipase. It is possible that in a few years the enzymatic catalysis will be economically feasible for biodiesel production on a large scale. The summary of recent studies using enzymatic transesterification process for biodiesel production can be observed in Table 3.

5. Membrane reactors

Reaction and separation are conducted on different stages of the process with different equipment in most chemical processes using continuous stirred-tank reactor (CSTR), plug-flow reactor (PFR), batch reactor and distillation column with diverse configuration [3,73–76]. Some reactive-separation technologies have been studied as possible alternatives with low capital investment and reduced operational cost for continuous operating in biodiesel production. Some reactors have been designed and used as a single device for reaction and separation in transesterification: centrifugal contactor separators [73], reactive distillation [77], reactive absorption [78], reactive extraction [79] and membrane

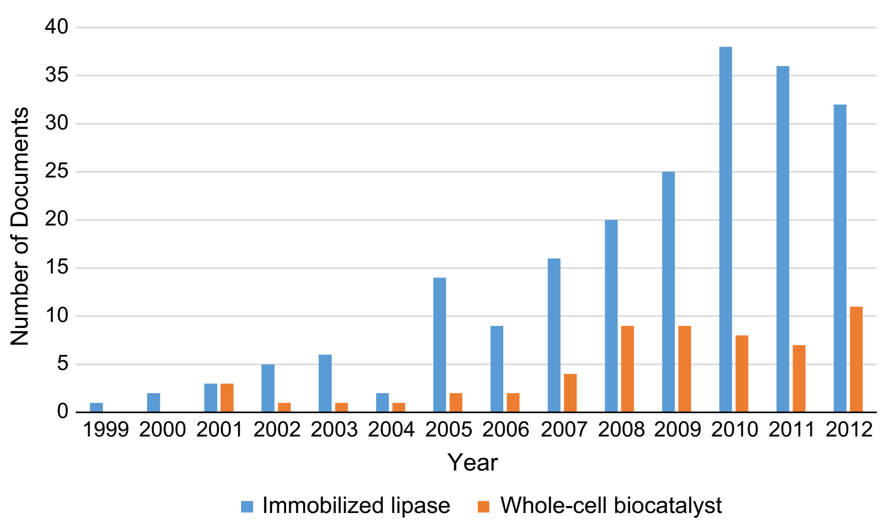


Fig. 2. Number of documents published for biodiesel produced by enzymatic catalysis [115].

Table 3
Resume of recent studies of enzymatic transesterification for biodiesel production.

Reference	Catalyst/oil	Immobilization support	Temperature (°C)	Yield (%)	Time reaction (h)
[70]	*C. antarctica* lipase/shirashime oil	Macroporous acrylic resin	30	98.9	–
[67]	*C. antarctica* lipase/high oleic sunflower	Lewatit VP OC	60	96.5	49
[69]	*P. cepacia* lipase/soybean	Fe_3O_4 nanoparticle in cotton	40	100	24
[54]	Recombinant *E. coli* cell/vegetable oils	–	15	100	12
[58]	Recombinant *A. oryzae* cell/soybean	Reticulated polyurethane foam	30	98	80

reactors [80] are some examples. This process integration is aimed on improving the productivity, with energy reduction, excluding the need for solvents generating an efficient process with green engineering characteristics [76,81].

The membrane reactor was defined by Cao, Tremblay [82] as a system which combines membrane separation and chemical reactions. It is used to simultaneously perform a reaction and membrane separation of products in the same operation unit. This approach permits to solving the equilibrium reaction limitation, enhancing mass and heat transfer conversion rate, besides the possibility of application in continuous mode operation. Furthermore, the membrane has the ability to function as a support for the solid catalyst (heterogeneous) avoiding an additional step of separating the additional catalyst. In the conventional process, a large amount of water used in the neutralization of alkaline catalyst purification and alkyl esters creates a step of treating waste water, which is the main problem in the process of homogeneous catalysis [83].

The membrane technology has been used in various processes of biorefining and bioenergy production, including: separation and purification of individual molecules from biomass, removal of fermentation inhibitors, enzyme recovery from hydrolysis processes, membrane bioreactors for bioenergy and chemical production, such as bioethanol, biogas and acetic acid, bioethanol dehydration, bio-oil and biodiesel production, and algae harvesting [84].

The application of membrane reactors can be based on three different principles. Based on the size of the oil droplets, the catalytic membrane, pervaporation. The application of catalytic membrane can be combined with catalyst in two ways: by integrating a catalyst and without incorporating of catalyst [9]. For the production of biodiesel membrane technologies are used based on principles of oil droplet size and catalytic membrane. The catalytically active membrane appears to be the best option for the production of biodiesel because fewer purification is necessary since catalytically inert membrane requires an additional purification due to the presence of a mixture of glycerol, methanol catalysts and FAME in the permeate flow.

To control the membrane reactor, it is necessary to know the variables with the most significant effects for the process. The parameters to be controlled are effect of reaction temperature, the alcohol to oil ratio, catalyst concentration, reactant flow rate, transmembrane pressure (TMP), the pore size and membrane thickness [9]. There are some physical–chemical characteristics of membrane that permits its application in biodiesel transesterification. The membrane offers a barrier to lipophilic substances present in lipid feedstocks. This difference offers reliability in the production of biodiesel that parallels the use of distillation in petroleum processes. The vapor–liquid interface determines the mass transfer mechanism in distillation, like the lipophilic–hydrophilic phase boundary does in the membrane reactor. The membrane serves to retain the smallest lipophilic droplets within the reactor considering the oil droplet principle [82].

The material characteristic of inorganic ceramic membranes offers resistance to chemical attack and thermal stability [85]. Resistance to corrosion is considered fundamental when the system is operated with base or acid catalysts.

In the conversion of vegetable oil by the transesterification process, the reversible reaction between the reactant and the product designates that the conversion for biodiesel is greatly dependent on the proportion of reactants and the conditions of the transesterification process. According to Le Chatelier's principle, big concentration of alcohol is necessary to switch the reaction equilibrium for the product side and rise the conversion yield [9,86].

The most recent studies on membrane reactors for biodiesel have focused on the optimization of process parameters to improve the performance of the reactor. Some implementation strategies are used in addition to investigation of mechanisms associated with the thermodynamic phase equilibrium of ternary mixture between alcohol, triglycerides and alkyl esters. Cheng and Yen [87] studied the effects of operating parameters, including methanol-to-oil molar ratio, catalyst concentration, temperature and the reaction time course for the reactant composition in the oil–FAME–MeOH ternary phase diagram. The results obtained indicate that increasing the residence time of the whole system within the two-phase zone improves the separation through membranes.

Studies to improve the recovery of methanol through the knowledge of the variation of process parameters are also performed and utilized to optimize the performance of membrane reactors. Baroutian and Aroua [88] studied the methanol recovery by means of continuous distillation after membrane separation and concluded that operational parameters including heating temperature, permeate flow rate and reactants ratio have significant effects on the rate of methanol recovery.

Falahati and Tremblay [89] studied the effect of membrane flux and residence time on the membrane reactor performance for different feedstock. Low free fatty acid (FFA) oils (FFA $<$ 1%), *i.e.*, canola, corn, sunflower and unrefined soy oils, and high FFA waste cooking oil (FFA = 5%) were alkali transesterified. The membrane reactor could be operated at the upper limit of the flux tested (70 L/m^2/h) and a residence time of 60 min.

Some authors have been studying the use of membranes as a downstream process in a separation unit only. The reaction is carried on in a batch reactor and separated by membrane filtration. Reyes et al. [8] developed a new semi-continuous strategy to produce and refine FAME at a low methanol-to-oil molar ratio using a ceramic membrane filtration process. The sequential batch coupled with the membrane reactor (SBMR) strategy was based on the operation of consecutive reactions and refining cycles; the latter operated only when a 70% FAME conversion yield was reached. This permitted an operation with a high permeate flux, due to the low viscosity of FAME compared to vegetable oil. The use of a stoichiometric methanol-to-oil molar ratio in the transesterification increases the accumulation of mono and diglycerides in a conventional batch reactor (CBR). However, this conventional batch reactor coupled with a membrane system (CBR–MS) allows the permeation of these compounds. The SBMR separated 99% of glycerol and reduced 79% and 78% of mono and diglycerides in FAME, respectively, compared to CBR. Therefore, the phase equilibrium during transesterification is a main factor to be considered in the implementation of a FAME separation–refining process, since the ceramic membrane is not able to remove MG and DG, but separation of these compounds is possible using the adequate operational strategy.

The evaluation of membrane performance and its behavior in transesterifcation of biodiesel have been studied in different approaches. For this purpose, a mathematical simulation of the operation of transesterification has been a brew of crucial importance to determine the merits of each approach or operational configuration. Cheng et al. [90] studied the modeling, simulation and experimental validation of biodiesel process using a membrane reactor integrated with a prereactor. The mathematical modeling considered and included the equations of transesterification kinetics, phase equilibrium, mass balance for both prereactor and tubular ceramic membrane derived from mass balances of both the feed side and the permeate side, attached with the mass transfer across the membrane. It was evaluated the integrated reactor performance according to the permeated biodiesel flux, selectivity of methanol-to-oil ratio in the feed, the initial reaction time in the prereactor, the volume ratio of the prereactor to tube membrane and the tube membrane length. The results showed

that the prereactor can be used for the purpose of carrying out a substantial part of the transesterification reaction in the early stage. The subsequent membrane reactor could separate the unreacted emulsified oil from the product stream. The process was validated experimentally and fit considerably well with the simulated data by adjusting the operating conditions, including the initial reaction time in the prereactor and the tube membrane length.

The biodiesel production by catalytic membrane reactor is a new technology which can be an alternative to solve the actual limitations related to conventional biodiesel production processes. The technology is considered environmentally friendly with lower cost invest requirement, overcoming the limitation caused by the chemical equilibrium of the reaction, with high flexibility related to the type of raw material used, generating products according to international standards [9].

6. Process combination

Recent work involving the technologies mentioned in this article has been recently applied in combined mode in order to increase efficiency and optimize performance in the transesterification reaction for biodiesel production. This approach is based on the combination of the mechanisms involved for each technology. Microwave for heat transfer, ultrasound to mass transfer and membrane reactors for the process of simultaneous reaction and separation. All are applied to continuous operation mode in the transesterification reaction for biodiesel production with green process characteristics. In addition, some studies describe the possible application in enzymatic catalysis: microwave [91–93]; ultrasound [94–96] and membrane reactors [97,98] have been recently studied in combination with enzymatic catalysis for different products obtainment.

The possible combination of microwave irradiation and enzymatic transesterification for biodiesel transesterification was reported by Nogueira and Carretoni [99] and the results showed that the microwave method increased the lipase activity but the time exposure led to enzyme deactivation. Da Rós and Freitas [100] also combined enzymatic ethanolysis with a microwave heating system. Using palm oil and a *Pseudomonas fluorescens* lipase immobilized on an epoxy polysiloxane–polyvinyl alcohol hybrid composite, they obtained the optimal conditions ranging from 8 to 15 W (according to reaction temperature), 8:1 ethanol-to-oil molar ratio at 43 °C reaching 97.56% of palm oil conversion in a 12 h reaction, conforming to a six fold increase compared to the conventional heating assisted-process. This work showed that microwave irradiation accelerated the enzyme-catalyzed reaction and no destructive effects on the enzyme properties were observed, such as stability and substrate specificity. In addition, the microwave irradiation permits uniform heating of the entire reaction volume. The approach presented a low energy demand and a faster conversion of palm oil into biodiesel. The resume of the results is presented in Fig. 3

The enzymatic transesterification has also been combined with ultrasound irradiation for biodiesel production as Yu and Tian [101] studied the transesterification of soybean oil and methanol using Novozym 435 lipase under ultrasonic irradiation and vibration. The results showed that the combination of ultrasonic with vibration increased the lipase catalytic activity and improved the time of reaction, reaching a 96% yield of fatty acid methyl ester in 4 h.

Similar results were also obtained by Batistella and Lerin [102] who studied the enzymatic soybean oil ethanolysis in organic solvent under ultrasonic irradiation. The reaction with two commercial immobilized lipases in an ultrasonic bath was performed.

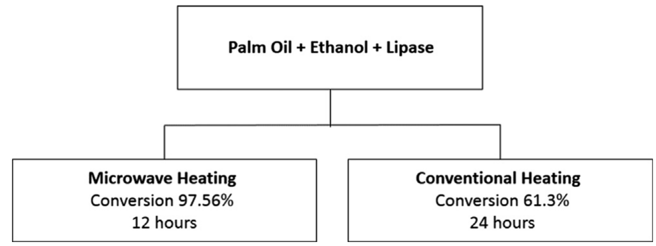


Fig. 3. Study developed by Da Rós and Freitas [100].

The results showed high reaction yields (~90 wt%) obtained at mild irradiation power supply (~100 W) and temperature (60 °C) in a relatively short reaction time, 4 h, using Lipozyme RM as catalyst. The use of Novozym 435 led to lower conversions (about 57%) nevertheless, the enzyme activity was stable after eight cycles of use, showing a reduction in product conversion after the fourth cycle.

Tupufia and Jeon [103] reported in coconut oil alcoholysis that the alkali reaction was about 2 orders of magnitude faster than the lipase reaction, however resulted in saponification/partial solidification. They also observed that the ratios of the kinetic constants during enzymatic transesterification are in agreement with reaction stoichiometry, resulting in purified products. The resume of the results are presented in Fig. 4.

Most studies report only the application of microwave or ultrasound. However, it is possible that these technologies can be applied in combined mode whereas the mechanisms of each one is different. Recently, Hsiao et al. [27] employed ultrasonic mixing and microwave irradiation to assist soybean oil alkaline transesterifcation with the objective to enhance the fatty acid ester yield and time reaction. Results showed that the optimal time of reaction was 1 min of ultrasonic mixing and 2 min of microwave irradiation. The optimal process conditions for 97.7% of conversion rate were: catalyst concentration, 1.0 wt%; reaction temperature, 60 °C and methanol:oil molar ratio, 6:1. The protocol established results in a 3-min reaction. The authors suggested that the ultrasonic mixing improved the performance of microwave irradiation which can be considered a synergistic effect between the mechanism of mass and heat transfer. The resume of results is presented in Fig. 5. Utilizing the same approach combining microwave and ultrasound in sequence for biodiesel synthesis, Gole and Gogate [104] transesterified the high value acid Nagchampa oil in a two-step synthesis method. The first step comprises of esterification for acid neutralization and the second, an alkali transesterification. Both steps carried out under microwave and ultrasound irradiation. The reaction time for the esterification and transesterification using ultrasound alone was 60 min and 20 min, respectively, and it reduced to only 15 min and 6 min for the sequential approach. The innovation is that the required excess of alcohol is significantly reduced (ratio 1:4), which can lead to a substantial energy saving in the downstream separation.

There are studies that report the application of these technologies combined with catalytic membranes and ultrafiltration membranes [105–107] for several bioprocesses. Zhang et al. [16] studied the performance of conventional heating and microwave-assisted method for biodiesel production using cation ion-exchange resin particles (CERP)/PES catalytic for transesterification of waste cooking oil (WCO). The experimental results showed that microwave irradiation exhibited a remarkable enhanced effect for esterification compared with that of the traditional heating method, reaching 97.4% under the optimal conditions of reaction temperature 60 °C, methanol/acidified oil mass ratio, 2:1, catalytic membrane (annealed at 120 °C) loading, 3 g, microwave power, 360 W and reaction time 90 min.

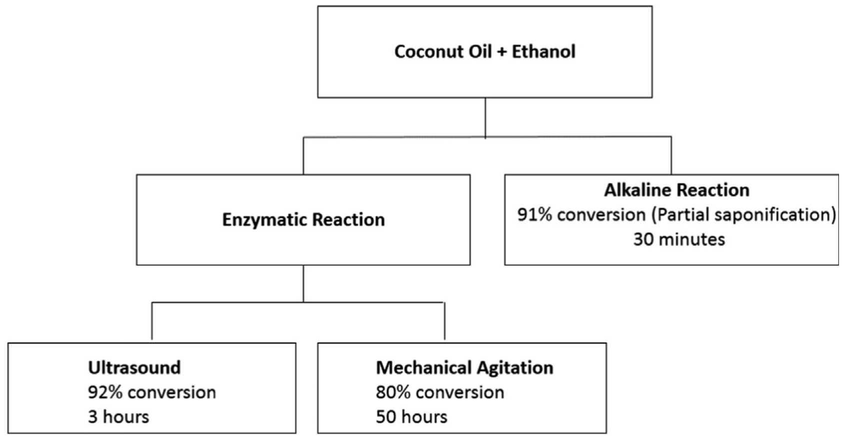


Fig. 4. Protocol of the study approach developed by Tupufia and Jeon [103].

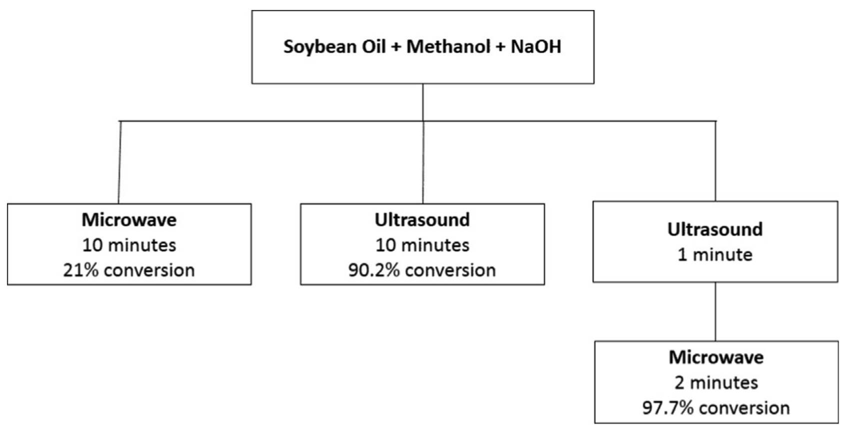


Fig. 5. Protocol study of alkaline alcoholysis approach developed by Hsiao and Lin [27].

Some studies have demonstrated the possibility of enzyme application as biocatalysts in membrane reactors. These biocatalytic membranes have been prepared with different methods of immobilization and the results show that it is possible the development of membrane bioreactors catalyzed by the enzyme. Badenes and Lemos [108] produced biodiesel through the transesterification of oils with an alcohol using catalytic membrane bioreactor (MBR) with recombinant cutinase of *Fusarium solani pisi* microencapsulated in sodium bis (2-ethylhexyl) sulfo-succinate (AOT)/isooctane reverse micelles. A ceramic tubular membrane with a nominal molecular weight cutoff of 15 kDa was used to retain the enzyme. The MBR performance was tested in continuous operation and the total recirculation mode was compared to a batch stirred tank reactor. The results of comparison between the performances of cutinase wild-type and the mutant T179C showed that the second one have high operational stability in the long term, demonstrating potential for continuous production of biodiesel.

The combination of enzyme catalysis with membrane reactors has been investigated by an approach using the membrane as an ultrafiltration unit only and the reaction being made in continuous batch reactor. The aim was to study the production of biodiesel by applying a system to remove glycerol through a membrane bioreactor, thus avoiding the possible inhibition by glycerol. It was found that the inhibition by methanol can be reduced by continuously feeding methanol in the membrane system and the membrane bioreactor system may be used efficiently for the biodiesel production [109].

The possible combination of ultrasound, microwave, enzymatic catalysis and membrane reactors have been recently published with results showing potential to enhance reaction performance, besides the possibility for application in continuous mode in biodiesel production. This improvement in performance can be attributed to the synergistic benefits obtained by summation of the effects of each technology establishing new trends for biodiesel process intensification with green engineering attributes.

7. Conclusion

Biodiesel is gradually gaining acceptance in the market as an environmentally friendly biofuel. However, for its establishment, various aspects need to be improved. Enhancing the efficiency of the production process using low cost feedstock and developing a cost-effective catalyst combined with physical processes have been reviewed and researched in order to design an efficient biodiesel process. Some parameters of the whole operation are important to define it as sustainable, such as the use of edible sources of feedstock, waste-cooking oil, alcohol, glycerol sales and residue reuse. These parameters need to be determined as environmentally safe for the sustainability of the whole process.

Physical technologies have improved the heat and the mass transfer and separation operations in bioprocesses and this combination may be a trend for green processes development due to its high efficiency and environmental harmlessness. The application of microwaves for heat transfer, ultrasound for mass transfer

and membrane reactors for simultaneous reaction–separation may be a practical improvement, considering the evolutions in intensification for industrial process. This present work showed which have been published recently in the combination of this physical technologies with enzymatic transesterification using lipases as biocatalysts with the possibility of an approach using an enzymatic membrane reactor coupled with a system of ultrasound and microwave. This experimental application for biodiesel production shows that its potential is real and that the results collaborate to reduce operating costs of the process, making the product more competitive and affordable for the market.

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